IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	Chomczynski
Serial No.	10/826,113
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Title	**REAGENTS AND METHODS FOR ISOLATION OF PURIFIED RNA**
Examiner	Fredman
Art Unit	1637
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Mail Stop AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF PIOTR CHOMCZYNSKI
PURSUANT TO 37 CFR §1.132

I, Piotr Chomczynski, Ph.D., declare as follows:

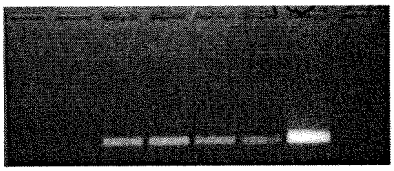
1. I am the inventor in the above-identified patent application.

2. I received my degree in Biological Sciences from the Polish Academy of Sciences. I have over 30 years of experience in molecular biology and genomic research, which is the subject of this application. This includes academic lecturing, participation in scientific conferences, authoring peer-reviewed scientific publications, and performing both basic and applied research. I have read the outstanding Office Action and understand the Examiner's position.

3. I disagree that Chen anticipates my claims 29-39, 41, 45-52, and 59-61, and that Chen in view of the secondary references renders obvious my claims 42 and 44, at least because my claimed method results in purified RNA, which I have defined as substantially undegraded and free of DNA contamination when assayed by RT-PCR. In contrast, Chen's method does not result in purified RNA.

4. I isolated total RNA from rat liver by each of:
(a) the reagent of my Example 1 (results shown in lanes 1 and 2),
(b) the reagent of Chen's embodiment 1 (results shown in lanes 3 and 4), and
(c) the reagent of Chen's embodiment 2 (results shown in lanes 5 and 6).

1 2 3 4 5 6 7 8



I analyzed the isolated RNA for DNA contamination. DNA contamination was detected using 0.2 µg of RNA in PCR with primers for the *cfos* gene fragment. After a 30-cycle reaction, an aliquot of the PCR mix was separated by electrophoresis in 1% agarose gel and stained with ethidium bromide. Lane 7 shows control PCR with rat DNA, and lane 8 shows control PCR without DNA.

5. The results demonstrated that Chen's reagents and method (lanes 3, 4, 5, and 6) is contaminated with DNA. This is not purified RNA that results from my method (lanes 1 and 2), as I have claimed and disclosed.

6. The yield of isolated RNA was as follows:

using Chen's reagent and following Chen embodiment 1, 5.9 µg RNA /mg tissue

using Chen's reagent and following Chen embodiment 2, 3.1 µg RNA /mg tissue

7. When the reagents and methods of Chen's Embodiment 1 and Chen's Embodiment 2 of were used in PCR with primers for the *cfos* gene, it amplified detectable amount of the *cfos* DNA fragment.

8. At least one reason that Chen's method does not result in purified RNA as I have claimed and defined it may be due to Chen's absolute requirement of a higher concentration of detergent. In the method recited in my claim 29 and claims depending therefrom, a detergent is optional and, if present, is at a lower concentration than Chen's, as I further explain.

9. Chen's method requires a detergent at a minimal concentration > 0.1% w/w, while in my method a detergent is optional and, if included, is present at a concentration < 0.1% w/w. Specifically, Chen's RNA extraction reagent "..compris[es] a highly effective inhibitor [guanidine isothiocyanate and guanidine hydrochloride, with water and phenol as dissolvent], scaling agent [i.e., detergent], pH regulator, and dissolvent of RNA enzymes." "In addition, there is also mercaptoethanol as the anti-oxidant in the present extraction reagent." (Examiner's translation p. 6, emphasis added). The pH is 3.5 to 6.5 (Examiner's translation p. 7). "The quality of the extracted RNA is slightly better than the imported TRI-zol reagent..." (Examiner's translation p. 10).

10. Chen's "scaling agent" (i.e., detergent) is present at a minimum concentration of 0.2% w/w and a maximum concentration of 0.7% w/w. A detergent is an integral part of Chen's RNA extraction reagent (Chen claims 1, 2, 5, and p. 6 second sentence, first full paragraph) (Chen's claims 2 and 5 define amount of scaling agents as 0.12% to 0.35% sodium dodecylsarcosinate and 0.1% to 0.31% sodium dodecylsulfate; thus, Chen's minimal concentration of detergents (scaling agents) is 0.21% w/w. In Chen's preferred embodiment 1 (Examiner's translation p. 7), the detergent concentration is much higher and amounts to 0.66%).

11. In contrast, a detergent is optional in my method of claim 29 and claims depending therefrom to isolate purified RNA and, if a detergent is present, it is at a lower concentration than Chen's (0.1% w/w; specifically Example 1, 01% w/w sarcosine, in Example 3, 0.1% w/w Triton X-100, and in Example 6, 0.05% w/w sarcosine).

12. At least one reason that Chen's method does not result in purified RNA as I have claimed and defined it may be due to Chen's very low concentration of buffer. Chen recites "0.005-0.02% pH regulator (see Chen claim 1), as I further explain.

13. Each of my claims 47 and 48 and claims depending therefrom requires "a buffer sufficient to maintain a pH of the composition in the range from about pH 3.6 to about pH 5.5". In contrast, Chen's "0.005-0.02% pH regulator" corresponds to a maximal concentration of about 2 mM sodium ions in Chen's reagent. This provides almost no buffering capacity.

14. Chen's buffering capacity would not be sufficient to maintain a pH from about 3.6 to about 5.5, as each of these claims require.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the subject application or any patent issued thereon.

1-17-07
Date

Piotr Chomczynski
Piotr Chomczynski, Ph.D